

Amendments to the Claims

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1. (Currently Amended) An electrophoresis system for the separation of proteins, comprising:
 - (i) at least one low conductivity organic solvent buffer comprising at least one base solvent, at least one conductivity enhancer, and ~~optionally~~ at least one conductivity suppressor;
 - (ii) a polymeric membrane having high-protein binding capacity, which membrane is compatible with the at least one low conductivity organic solvent buffer; and
 - (iii) an electrophoresis apparatus which comprises at least one electrophoresis unit for containing the buffer and membrane, and a power supply capable of generating an electric current in the at least one electrophoresis unit wherein said at least one electrophoresis unit comprises
 - (1) a platform on which the membrane is placed wherein said membrane is no more than coextensive in length and width than said platform; and
 - (2) at least one wick having a first and a second end, said at least one wick disposed below and in direct contact with said membrane wherein at least the first end of said at least one wick is in contact with the buffer.
2. (Original) The electrophoresis system of claim 1, wherein the at least one base solvent is present in the low conductivity organic solvent buffer in a final concentration of about 1% to about 80%.

3. (Original) The electrophoresis system of claim 1, wherein the at least one base solvent is present in the low conductivity organic solvent buffer in a final concentration of about 20% to about 50%.
4. (Original) The electrophoresis system of claim 1, wherein the at least one base solvent is selected from the group consisting of propylene carbonate; ethylene cyclic carbonate; dimethyl phthalate; diethyl phthalate; ethylene glycol; propylene glycol; butylene glycol; dimethyl sulfoxide; methyl carbitol; and mixtures thereof.
5. (Original) The electrophoresis system of claim 4, wherein the base solvent is propylene carbonate.
6. (Original) The electrophoresis system of claim 4, wherein the base solvent is ethylene cyclic carbonate.
7. (Original) The electrophoresis system of claim 4, wherein the base solvent is a mixture of propylene carbonate and ethylene cyclic carbonate.
8. (Original) The electrophoresis system of claim 1, wherein the at least one conductivity enhancer is present in the low conductivity organic solvent buffer in a final concentration of about 0.1% to about 50%.
9. (Original) The electrophoresis system of claim 1, wherein the at least one conductivity enhancer is present in the low conductivity organic solvent buffer in a final concentration of about 5% to about 30%.
10. (Original) The electrophoresis system of claim 1, wherein the at least one conductivity enhancer is selected from the group consisting of formamide; acetamide; propionamide; butyramide; N-methyl formamide; N-methyl acetamide; N-methyl propionamide; N-methyl butyramide; benzamide; toluamide; lactamide; nicotinamide; 2-furaldehyde; furfuryl alcohol; tetrahydrofurfuryl alcohol; salicylaldehyde; guaiacol; phenol; boric acid; fumaric acid; piperazine; and mixtures thereof.

11. (Original) The electrophoresis system of claim 10, wherein the at least one conductivity enhancer is a mixture of salicylaldehyde and furfuryl alcohol.
12. (Original) The electrophoresis system of claim 10, wherein the at least one conductivity enhancer is a mixture of formamide and 2-furaldehyde.
13. (Original) The electrophoresis system of claim 10, wherein at least one conductivity enhancer is a mixture of formamide and furfuryl alcohol.
14. (Original) The electrophoresis system of claim 10, wherein at least one conductivity enhancer is a mixture of formamide and tetrahydrofurfuryl alcohol.
15. (Original) The electrophoresis system of claim 1, wherein the low conductivity organic solvent buffer comprises at least one conductivity suppressor.
16. (Original) The electrophoresis system of claim 1, wherein the at the least one conductivity suppressor is present in the low conductivity organic solvent buffer in a final concentration of about 0.1% to about 50%.
17. (Original) The electrophoresis system of claim 1, wherein the at the least one conductivity suppressor is present in the low conductivity organic solvent buffer in a final concentration of about 5% to about 30%.
18. (Original) The electrophoresis system of claim 1, wherein the at least one conductivity suppressor is selected from the group consisting of dimethyl derivatives of formamide and acetamide; 1,3-butanediol; N-methyl pyrrolidinone; sorbitol; glycerol; caprolactone; methoxyethanol; and mixtures thereof.
19. (Original) The electrophoresis system of claim 18, wherein at the least one conductivity suppressor is a mixture of 1,3-butanediol, dimethyl formamide and dimethyl acetamide.
20. (Original) The electrophoresis system of claim 18, wherein the at least one conductivity suppressor is a mixture of 1,3-butanediol and N-methyl pyrrolidinone.

21. (Original) The electrophoresis system of claim 18, wherein the at least one conductivity suppressor is 1,3-butanediol.
22. (Original) The electrophoresis system of claim 1, wherein the at least one low conductivity organic solvent buffer has a pH of about pH 3 to about pH 10.
23. (Original) The electrophoresis system of claim 1, wherein the membrane is a hydrophobic membrane.
24. (Original) The electrophoresis system of claim 1, wherein the membrane is a hydrophilic membrane.
25. (Original) The electrophoresis system of claim 1, wherein the membrane binds at least about 20 μg protein/ cm^2 .
26. (Original) The electrophoresis system of claim 1, wherein the membrane binds at least about 50 μg protein/ cm^2 .
27. (Original) The electrophoresis system of claim 1, wherein the membrane binds 100 μg protein/ cm^2 to about 400 μg protein/ cm^2 .
28. (Original) The electrophoresis system of claim 23, wherein the hydrophobic membrane comprises a polymer selected from the group consisting of fluorinated polymers; polyolefins; polystyrene or substituted polystyrenes; polysulfones; polyethersulfones; polyesters; polyacrylates; polycarbonates; polyurethane; vinyl polymers; polyacrylonitriles; and mixtures thereof.
29. (Original) The electrophoresis system of claim 28, wherein the fluorinated polymer is polyvinylidene difluoride (PVDF).
30. (Original) The electrophoresis system of claim 28, wherein the polyolefins are polyethylene, polypropylene, or polymethylpentene.

31. (Currently Amended) The electrophoresis system of claim 28, wherein the polymer is a polyester and is selected from the group of polyesters consisting of~~polyesters are~~ polyethylene terephthalate and~~or~~ polybutylene terephthalate.
32. (Currently Amended) The electrophoresis system of claim 28, wherein the polymer is a vinyl polymer and said vinyl polymer is polyvinyl chloride.
33. (Original) The electrophoresis system of claim 23, wherein the hydrophobic membranes comprise butadiene-styrene copolymer or fluorinated ethylene-propylene copolymer.
34. (Original) The electrophoresis system of claim 24, wherein the hydrophilic membrane comprises a polymer selected from the group consisting of nylons; polyimides; polyesters; polyvinyl alcohols; polyvinylamines; polybenzylamides; polyvinylimidazolines; polydiallylamines; and mixtures thereof.
35. (Original) The electrophoresis system of claim 34, wherein the hydrophilic membrane comprises a nylon polymer.
36. (Original) The electrophoresis system of claim 35, wherein the nylon polymer has about 0.4 moles to about 2 moles amino end groups per mole of nylon.
37. (Original) The electrophoresis system of claim 1, wherein the membrane is about 0.01mm thick to about 3 mm thick.
38. (Original) The electrophoresis system of claim 37, wherein the membrane is about 0.10 mm thick to about 0.5 mm thick.
39. (Original) The electrophoresis system of claim 1, wherein the electrophoresis apparatus comprises a plurality of electrophoresis units.
40. (Original) The electrophoresis system of claim 1, wherein the at least one electrophoresis unit is a vertical electrophoresis unit.

41. (Original) The electrophoresis system of claim 1, wherein the at least one electrophoresis unit is a horizontal electrophoresis unit.
42. (Canceled)
43. (Currently Amended) A method for the electrophoretic separation of proteins, comprising the steps of:
- (1) providing at least one low conductivity organic solvent buffer comprising at least one base solvent, at least one conductivity enhancer, and ~~optionally~~ at least one conductivity suppressor;
 - (2) providing a polymeric membrane having high-protein binding capacity, which membrane is compatible with the at least one low conductivity organic solvent buffer;
 - (3) applying at least one sample comprising proteins to be separated to the membrane; and
 - (4) separating the proteins by electrophoresis utilizing an electrophoresis apparatus which comprises at least one electrophoresis unit for containing the buffer and membrane, and a power supply capable of generating an electric current in the at least one electrophoresis unit wherein said at least one electrophoresis unit comprises
 - (1) a platform on which the membrane is placed wherein said membrane is no more than coextensive in length and width than said platform; and
 - (2) at least one wick having a first and a second end, said at least one wick disposed below and in direct contact with said membrane wherein at least the first end of said at least one wick is in contact with the buffer.

44. (Original) The method of claim 43, wherein the proteins are separated in a first dimension.
45. (Original) The method of claim 43, wherein the proteins are separated in first dimension, and subsequently separated in a second dimension.
46. (Original) The method of claim 43, wherein the electrophoresis is performed under non-denaturing conditions.
47. (Original) The method of claim 46, further comprising the step of detecting enzymatic activity in the separated proteins.
48. (Original) The method of claim 47, wherein the enzymatic activity is detected with a colorimetric or a fluorogenic substrate.
49. (Original) The method of claim 46, further comprising the step of detecting protein-binding interactions in the separated proteins.
50. (Original) The method of claim 49, wherein an antibody is added to the sample before electrophoresis.
51. (Original) The method of claim 50, wherein the antibody is labeled with a detection agent.
52. (Original) The method of claim 51, wherein the detection agent is selected from the group consisting of colored dyes; fluorescent dyes; chemiluminescent labels; biotinylated labels; radioactive labels; affinity labels; and enzyme labels.
53. (Original) The method of claim 49, wherein the protein-binding interactions comprise protein-ligand interactions.
54. (Original) The method of claim 53, wherein a ligand is added to the sample before electrophoresis.

55. (Original) The method of claim 54, wherein the ligand is labeled with a detection agent.
56. (Original) The method of claim 55, wherein the detection agent is selected from the group consisting of colored dyes; fluorescent dyes; chemiluminescent labels; biotinylated labels; radioactive labels; affinity labels; enzyme labels; and protein-specific antibodies.
57. (Original) The method of claim 43, further comprising the step of detecting the separated proteins with at least one antibody.
58. (Original) The method of claim 57, wherein the antibody is labeled with a detection agent.
59. (Original) The method of claim 58, wherein the detection agent is selected from the group consisting of colored dyes; fluorescent dyes; chemiluminescent labels; biotinylated labels; radioactive labels; affinity labels; and enzyme labels.
60. (Original) The method of claim 59, further comprising the step of detecting protein-binding interactions in the separated proteins.
61. (Original) The method of claim 60, wherein the protein-binding interactions comprise the formation of protein-protein complexes.
62. (Original) The method of claim 49, wherein protein-binding interactions in the separated proteins are detected by a mobility shift of at least one of the separated proteins.
63. (Original) The method of claim 43, wherein the current generated in the electrophoresis unit is about 0.0001 mA/cm^2 membrane to about 0.2 mA/cm^2 membrane.
64. (Original) The method of claim 43, wherein the current generated in the electrophoresis unit is about 0.0005 mA/cm^2 membrane to about 0.05 mA/cm^2 membrane.
65. (Original) The method of claim 43, wherein the current generated in the electrophoresis unit is about 0.001 mA/cm^2 membrane to about 0.025 mA/cm^2 membrane.

66. (Original) The method of claim 43, wherein the electrophoresis unit is a horizontal electrophoresis unit.

67. (Original) The method of claim 43, wherein the at least one protein sample comprises a plurality of samples taken at different time points from a protein-containing preparation.

68. (Original) The method of claim 67, wherein the electrophoresis produces a degradation profile for the protein-containing preparation.

69. (Original) The method of claim 43, wherein the at least one protein sample is obtained from an organism at least two different time points.

70. (Original) A method for the two-dimensional electrophoretic separation of proteins, comprising the steps of:

- (1) providing an electrophoresis system comprising:
 - (i) a first low conductivity organic solvent buffer having a first pH and a second low conductivity organic solvent buffer having a second pH;
 - (ii) a membrane having a high protein binding capacity and which is compatible with the first and second organic solvent buffers; and
 - (iii) an electrophoresis apparatus which comprises at least one electrophoresis unit for containing the first and second organic solvent buffers and membrane;
- (2) applying at least one sample comprising proteins to be separated to the membrane;

- (3) placing the membrane and the first organic solvent buffer in the at least one electrophoresis unit, wherein the membrane is placed in a first orientation;
- (4) separating the proteins in a first dimension by generation of an electric current in the at least one electrophoresis unit;
- (5) replacing the first organic solvent buffer in the at least one electrophoresis unit with the second organic solvent buffer;
- (6) placing the membrane in the at least one electrophoresis unit in a second orientation; and
- (7) separating the proteins in a second dimension by generation of an electric current in the at least one electrophoresis unit.

71. (Original) The method of claim 70, wherein the membrane is washed to remove the first organic solvent buffer after separating the proteins in a first dimension.

72. (Original) The method of claim 70, wherein the first and second organic solvent buffers have the same composition.

73. (Original) The method of claim 70, wherein the electrophoresis unit is a horizontal electrophoresis unit.

74. (Original) The method of claim 70, wherein the electrophoresis apparatus comprises a plurality of electrophoresis units.

75. (Original) The method of claim 70, wherein the two-dimensional electrophoresis is performed under non-denaturing conditions.

76. (Original) The method of claim 75, further comprising the step of detecting enzymatic activity in the separated proteins.
77. (Original) The method of claim 75, further comprising the step of detecting protein-binding interactions in the separated proteins.
78. (Original) The method of claim 77, wherein the protein-binding interactions comprise the formation of protein-protein complexes.
79. (Original) The method of claim 78, further comprising the step of identifying the proteins in the protein-protein complexes.
80. (Original) The method of claim 77, wherein the protein-binding interactions comprise protein-ligand interactions.
81. (Original) The method of claim 77, wherein protein-binding interactions in the separated proteins are detected by a mobility shift of at least one of the separated proteins.
82. (Original) The method of claim 70, further comprising the step of detecting the separated proteins with at least one antibody.
83. (Currently Amended) The method of claim 43A method for the electrophoretic separation of proteins according to their isoelectric points, wherein the electrophoresis is performed in the absence of a pH gradient comprising the steps of:
- (1) ~~providing at least one low conductivity organic solvent buffer comprising at least one base solvent, at least one conductivity enhancer, and optionally at least one conductivity suppressor;~~
 - (2) ~~providing a polymeric membrane having high protein binding capacity, which membrane is compatible with the at least one low conductivity organic solvent buffer;~~

~~(3) — applying at least one sample comprising proteins to be separated to the membrane; and~~

~~(4) — separating the proteins by electrophoresis, wherein the electrophoresis is performed in the absence of a pH gradient.~~

84. (Original) The method of claim 83, wherein the proteins are separated in a first dimension.

85. (Original) The method of claim 83, wherein the proteins are separated in first dimension, and subsequently separated in a second dimension.

86. (Original) The method of claim 83, wherein the electrophoresis is performed under non-denaturing conditions.

87. (Original) The method of claim 86, further comprising the step of detecting enzymatic activity in the separated proteins.

88. (Original) The method of claim 86, further comprising the step of detecting protein-binding interactions in the separated proteins.

89. (Original) The method of claim 88, wherein the protein-binding interactions comprise the formation of protein-protein complexes.

90. (Original) The method of claim 89, further comprising the step of identifying the proteins in the protein-protein complexes..

91. (Original) The method of claim 88, wherein the protein-binding interactions comprise protein-ligand interactions.

92. (Original) The method of claim 88, wherein protein-binding interactions in the separated proteins are detected by a mobility shift of at least one of the separated proteins.

93. (New) An electrophoresis system for the separation of proteins, comprising:

- (i) at least one low conductivity organic solvent buffer comprising at least one base solvent, at least one conductivity enhancer, and at least one conductivity suppressor, wherein the at least one conductivity suppressor is selected from the group consisting of dimethyl derivatives of formamide and acetamide; 1,3-butanediol; N-methyl pyrrolidinone; sorbitol; glycerol; caprolactone; methoxyethanol; and mixtures thereof;
- (ii) a polymeric membrane having high-protein binding capacity, which membrane is compatible with the at least one low conductivity organic solvent buffer; and
- (iii) an electrophoresis apparatus which comprises at least one electrophoresis unit for containing the buffer and membrane, and a power supply capable of generating an electric current in the at least one electrophoresis unit.

94. (New) The electrophoresis system of claim 93, wherein the at least one low conductivity organic solvent buffer has a pH of about pH 3 to about pH 10.

95. (New) The electrophoresis system of claim 93, wherein the membrane is a hydrophobic membrane.

96. (New) The electrophoresis system of claim 93, wherein the membrane is a hydrophilic membrane.

97. (New) The electrophoresis system of claim 95, wherein the hydrophobic membrane comprises a polymer selected from the group consisting of fluorinated polymers; polyolefins; polystyrene or substituted polystyrenes; polysulfones; polyethersulfones; polyesters; polyacrylates; polycarbonates; polyurethane; vinyl polymers; polyacrylonitriles; and mixtures thereof.

98. (New) The electrophoresis system of claim 96, wherein the hydrophilic membrane comprises a polymer selected from the group consisting of nylons; polyimides; polyesters; polyvinyl alcohols; polyvinylamines; polybenzylamides; polyvinylimidazolines; polydiallylamines; and mixtures thereof.

99. (New) A method for the electrophoretic separation of proteins, comprising the steps of:

(1) providing at least one low conductivity organic solvent buffer comprising at least one base solvent, at least one conductivity enhancer, and, optionally, at least one conductivity suppressor, wherein the at least one base solvent is selected from the group consisting of

ethylene cyclic carbonate, dimethyl phthalate, diethyl phthalate, ethylene glycol, butylene glycol, dimethyl sulfoxide, methyl carbitol, and mixtures thereof; and

a mixture of propylene carbonate and one or more base solvents selected from the group consisting of ethylene cyclic carbonate, dimethyl phthalate, diethyl phthalate, ethylene glycol, butylene glycol, dimethyl sulfoxide, methyl carbitol; and

a mixture of propylene glycol and one or more base solvents selected from the group consisting of ethylene cyclic carbonate, dimethyl phthalate, diethyl phthalate, ethylene glycol, butylene glycol, dimethyl sulfoxide, methyl carbitol;

(2) providing a polymeric membrane having high-protein binding capacity, which membrane is compatible with the at least one low conductivity organic solvent buffer;

(3) applying at least one sample comprising proteins to be separated to the membrane; and

(4) separating the proteins by electrophoresis.

100. (New) A method for the electrophoretic separation of proteins, comprising the steps of:

(1) providing at least one low conductivity organic solvent buffer comprising at least one base solvent, at least one conductivity enhancer, and, optionally, at least one conductivity suppressor wherein the at least one conductivity enhancer is selected from the group consisting of:

formamide; acetamide; propionamide; butyramide; N-methyl formamide; N-methyl propionamide; N-methyl butyramide; benzamide; toluamide; lactamide; nicotinamide; 2-furaldehyde; furfuryl alcohol; salicylaldehyde; guaiacol; phenol; boric acid; fumaric acid; piperazine; and mixtures thereof; and

a mixture of N-methyl acetamide and one or more conductivity enhancers selected from the group consisting of formamide; acetamide; propionamide; butyramide; N-methyl formamide; N-methyl propionamide; N-methyl butyramide; benzamide; toluamide; lactamide; nicotinamide; 2-furaldehyde; furfuryl alcohol; salicylaldehyde; guaiacol; phenol; boric acid; fumaric acid; piperazine; and

a mixture of tetrahydrofurfuryl alcohol and one or more conductivity enhancers selected from the group consisting of formamide; acetamide; propionamide; butyramide; N-methyl formamide; N-methyl propionamide; N-methyl butyramide; benzamide; toluamide; lactamide; nicotinamide; 2-furaldehyde; furfuryl alcohol; salicylaldehyde; guaiacol; phenol; boric acid; fumaric acid; piperazine;

- (2) providing a polymeric membrane having high-protein binding capacity, which membrane is compatible with the at least one low conductivity organic solvent buffer;
- (3) applying at least one sample comprising proteins to be separated to the membrane; and
- (4) separating the proteins by electrophoresis.

101. (New) A method for the electrophoretic separation of proteins, comprising the steps of:

- (1) providing at least one low conductivity organic solvent buffer comprising at least one base solvent, at least one conductivity enhancer, and at least one conductivity suppressor selected from the group consisting of dimethyl derivatives of formamide and acetamide; 1,3-butanediol; N-methyl pyrrolidinone; sorbitol; glycerol; caprolactone; methoxyethanol; and mixtures thereof;
- (2) providing a polymeric membrane having high-protein binding capacity, which membrane is compatible with the at least one low conductivity organic solvent buffer;
- (3) applying at least one sample comprising proteins to be separated to the membrane; and
- (4) separating the proteins by electrophoresis.

102. (New) An electrophoresis system for the separation of proteins, comprising:

- (i) at least one low conductivity organic solvent buffer comprising at least one base solvent, at least one conductivity enhancer, and optionally at least one conductivity suppressor;

- (ii) a polymeric membrane having high-protein binding capacity, which membrane is compatible with the at least one low conductivity organic solvent buffer; and
- (iii) an electrophoresis apparatus which comprises at least one horizontal electrophoresis unit for containing the buffer and membrane, and a power supply capable of generating an electric current in the at least one horizontal electrophoresis unit, wherein the at least one horizontal electrophoresis unit comprises:
 - (1) a first and a second independent buffer chamber;
 - (2) a top plate and a bottom plate of substantially similar length and width bridging the first and a second independent buffer chambers, wherein the membrane is sandwiched between the top and bottom plates; and
 - (3) a wick with a first and a second end disposed between the bottom plate and the membrane, wherein the wick is longer than the top and bottom plates such that the first and second wick ends extend into the first and a second independent buffer chambers, respectively.

103. (New) The electrophoresis system of claim 1 wherein the second end of said at least one wick is in contact with the buffer.

104. (New) The electrophoresis system of claim 1 further comprising a second wick.

105. (New) A method for the electrophoretic separation of proteins, comprising the steps of:

- (1) providing at least one low conductivity organic solvent buffer comprising at least one base solvent, at least one conductivity enhancer, and at least one conductivity suppressor;

- (2) providing a polymeric membrane having high-protein binding capacity, which membrane is compatible with the at least one low conductivity organic solvent buffer;
- (3) applying at least one sample comprising proteins to be separated to the membrane, wherein the at least one sample is mixed with a wetting agent comprising ϵ -caprolactone; and
- (4) separating the proteins by electrophoresis.